

# Soil Microbial Activity and Diversity in Response to Soil Chemical Factors in Agricultural Soils

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## ABSTRACT

The role of microbial communities in maintaining soil health is mostly influenced by chemical condition of soil. Microbial communities vary in response to soil chemical factors. The contradictory results from previous findings emphasise that it is difficult to define a pattern of the influence of soil chemical factors on soil microbial diversity and activity. The aim of the study was to assess soil microbial responses to soil chemical factors in agricultural soils. Composite soil (Dermosol order) samples taken from 16 commercial crop sites in Bundaberg, Queensland, Australia, were chemically and biologically analysed. It was found that bacterial and fungal activity and diversity were significantly affected by soil EC, SOM and NO<sub>3</sub>-N content, but were not influenced by soil pH, CEC, and Ca:Mg ratio. The diversity of bacterial and fungal communities displayed a positive linear relationship with soil EC, whereas the activity and diversity of these two microbial groups and SOM displayed a significant quadratic relationship. The finding suggested that microbial community was predominantly influenced by SOM content.

**Keywords:** Agricultural soils, CLPP, soil chemical factors, soil microbial activity and diversity

## ABSTRAK

Peran komunitas mikroba dalam memelihara kesehatan tanah dipengaruhi oleh kondisi kimia tanah. Respon komunitas mikroba terhadap faktor-faktor kimia tanah sangat bervariasi. Beberapa penelitian sebelumnya menunjukkan hasil yang kontradiktif, sehingga sulit mendefinisikan suatu pola pengaruh faktor kimia tanah terhadap aktivitas dan keragaman mikroba tanah. Tujuan dari penelitian ini adalah untuk menganalisis respon dari mikroba tanah terhadap faktor-faktor kimia tanah pada tanah-tanah pertanian. Sampel tanah dari ordo Dermosol diambil secara komposit dari 16 lokasi pertanian di wilayah Bundaberg, Queensland, Australia, untuk dianalisis komponen kimia dan biologinya. Hasil penelitian menunjukkan bahwa aktivitas dan keragaman bakteri dan cendawan sangat dipengaruhi oleh konduktivitas elektrik tanah, bahan organik tanah (BOT), dan NO<sub>3</sub>-N tanah, namun lemah sekali atau tidak dipengaruhi oleh pH tanah, kapasitas tukar kation, dan rasio Ca:Mg tanah. Keragaman komunitas bakteri dan cendawan menunjukkan hubungan linear positif dengan konduktivitas elektrik, sementara aktivitas dan keragaman kedua kelompok mikroba ini menunjukkan hubungan kuadrat dengan BOT. Hasil penelitian menyimpulkan bahwa komunitas mikroba tanah sangat dipengaruhi oleh kandungan BOT.

## INTRODUCTION

Bacterial and fungal functional groups play an important role in a wide range of soil functions including soil-borne disease suppression (Nannipieri and Badalucco 2003; Coleman *et al.* 2004). As

soil health is manifest of the maintenance of soil functions (Lal 2011), the role of soil microbial communities in the maintenance of soil health cannot be ignored. This implies that the capacity of healthy soils to sustain soil biota and plant life through the maintenance of a wide range of soil biochemical processes and soil-borne disease suppression (Doran and Zeiss 2000) depends on the viability of soil microbial communities, and their diversity and

activity (Lange *et al.* 2014). Yet, the size, activity and diversity of microbial community is influenced by soil factors including soil chemical components (Brennan and Acosta-Martinez 2017).

Soil chemical factors are thought to influence diversity of soil microbial communities and their activity in disease suppression (Haggag 2002; Heyman *et al.* 2007). Studies on the effects of soil chemical factors on microbial community have included investigation of components including SOM, soil C and N, and pH which are regarded as the most important factors influencing the activity and diversity of soil microbial community (Cookson *et al.* 2008; Lauber *et al.* 2008; Jordi Garcia-Pausas and Paterson 2011; Lupwayi *et al.* 2012). In relation to nutrient effects, several studies have found that soil microbial activity is associated with the availability of calcium (Ca) particularly in regulating disease suppression, in which Ca concentration in soil showed a strong negative correlation with disease onset (Falcon, Fox and Trujillo 1984; Heyman *et al.* 2007). Yet, the availability of Ca and other nutrients like P are pH limited (Falcon *et al.* 1984), so interactions between soil pH and nutrient effects are likely to make interpretation of data quite complex. In addition, soils high in fertility and nutrient availability favour bacterial community, while low fertility and nutrient availability favour fungal community (Grayston *et al.* 2004). However, contradictory results of studies have been published (Fierer and Jackson 2006; Stark *et al.* 2008; Tu *et al.* 2014), suggesting the characteristics of soil chemical factors and microbial community relationships are still poorly understood.

The effects of soil chemical factors on soil microbial communities are likely to be very complex (Whalley *et al.* 1995). In order to gain deeper understanding on the relationships between these two soil components, the present study was undertaken to assess the differences in response of soil microbial communities to different chemical properties of agricultural soils. It was hypothesised that different soil microbial communities would inhabit soils with different chemical properties.

## MATERIALS AND METHODS

### Field Investigation and Study Sites

An investigation to examine the relationship between soil microbial activity and diversity and soil chemical factors is required to provide further information on the changes of soil microbial activity and diversity in soils under different chemical characteristics. Soil chemical factors chosen for this

investigation are soil pH, electrical conductivity (EC), soil organic matter (SOM), nitrate ( $\text{NO}_3\text{-N}$ ), cation exchange capacity (CEC), and calcium:magnesium ratio (Ca:Mg), whilst soil microbial properties are indicated by catabolic activity and functional diversity.

The experiment involved collection of soil samples from 16 commercial crop sites in the Bundaberg region, QLD, Australia, in April 2015. The soils were classified by Australian Soil Classification as Dermosol (Isbel 1997). Samples were taken from the field that had capsicum crops grown in rotation with other vegetable crops for one or more years. At each site, soil samples were collected from the root zone of capsicum plants at 15 cm depth with a soil corer (5 cm in diameter) and bulked.

### Soil Chemical Analyses

Soil chemical properties of the soils from 16 locations were analysed in the laboratory. Total soil carbon (C) content and total soil nitrogen (N) content were analysed after dry combustion using a CNS-2000 analyser (LECO Corporation, St. Joseph, MI, USA). Soil organic matter content was calculated by multiplying the percentage of total C with 1.724 factor (Nelson and Sommers 1982). Soil pH was measured using a suspension of 1:5 soil:water ratio (McLean 1982). Electrical conductivity was determined using a suspension of 1:5 soil:water ratio (McLean 1982). Cation exchange capacity and exchangeable cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^{+}$ , and  $\text{Na}^{+}$ ) were analysed with ammonium acetate method (1N  $\text{NH}_4\text{OAc}$  at pH 7.0) (Thomas 1982).

### Soil Biological Assessment

Soil biological properties were analysed using community level physiological profiles (CLPP) method. Soil bacterial and fungal activity and diversity were assessed to measure soil microbial responses to the effect of soil chemical characteristics from 16 different locations. Soil bacterial activity and diversity was analysed using the Biolog Eco-Plates™, whilst the Biolog FF-plate was utilised for analysing activity and diversity of fungal community. For the analysis of soil bacterial activity and diversity, three replicates per soil sample from 16 locations were used. Meanwhile, analysis of fungal activity and diversity was held for soil samples from 16 locations with one replicate per soil sample.

The CLPP method principally involves direct inoculation of soil samples into Biolog microtitre plates, incubation, and spectrometric detection on

heterotrophic microbial activity (Insam and Goberna, 2004). For microbial cell extraction, a 10 g fresh soil sample was suspended with a 100 mL of sterile saline solution (NaCl) (0.85%, w/v) in a 250 mL schott bottle. Saline solution was made by dissolving 4.25 g of NaCl in 500 ml of distilled water to prepare 0.85% saline solution and then sterilising the solution, together with all transfer equipment and glassware prior to use, in autoclave at 121°C for 16 min. The soil suspension was then shaken using a rotary shaker at 200 rpm for 1 h at a room temperature of 25°C. The suspension was allowed to settle for 11 h before inoculation to allow utilisation of any soluble organic compounds from the soil by soil microbia. Then, a 5 ml of the suspension was diluted 10-fold with 45 mL of sterile deionised water to make a 50 mL final volume. The final aliquot with 1000-fold dilution was used for inoculation. The protocols outlined above are also used for performing fungal extraction except the aliquots must contain antibiotics (10 µg streptomycin and 1.5 µg tetracycline per a 100 µL of a microtiter-plate well) to limit the growth of bacteria (modified from Classen *et al.* (2003). A 150 µL of 1000-fold diluted solutions were inoculated into each well of the Biolog Eco-Plates containing 31 different C sources and one non-substrate (water) solution in triplicates for bacterial

inoculations, while 100 µL per well was used in the FF plates containing 96 different carbon sources for fungal inoculation. After filling all the wells with the diluted solution, initial reading was taken using a spectrophotometer plate reader at 590 nm wavelength for bacterial Eco-Plates and 750 nm for fungal FF plates. The inoculated Eco-Plates were then incubated at 30°C and FF plates at 26°C in the dark. The microplates were monitored at 24 h intervals for 7 days (0, 24, 48, 72, 96, 120, 144, 168 h) for bacteria and fungi.

### Statistical Analyses

Regression analyses (linear and quadratic models) were used to determine any relationships between soil bacterial and fungal activity and diversity and soil chemical components in 16 locations. Analyses were performed using Minitab version 16.

## RESULTS AND DISCUSSION

### Soil Bacterial and Fungal Activity in Response to Soil Chemical Factors

Soil bacterial and fungal CLPP were compared among the treatments using several soil chemical

Table 1. Chemical and biological properties of soil samples taken from 16 different sites at the Bundaberg region, QLD.

Site	Soil chemical properties						Soil Biological properties			
	pH	EC (dS m <sup>-1</sup> )	SOM (%)	NO <sub>3</sub> -N (mg L <sup>-1</sup> )	CEC (meq 100g <sup>-1</sup> )	Ca:Mg ratio	Microbial activity		Microbial diversity	
							Bacteria (AWCD*)	Fungi (Turbidity)	Bacteria	Fungi
#1	6.0	0.12	5.52	24	9.99	2.47	1.73	0.42	3.32	3.1
#2	5.4	0.06	3.63	15	7.47	4.16	0.61	0.29	2.88	2.2
#3	6.4	0.09	12.17	11	23.46	2.05	0.52	0.02	2.71	1.9
#4	5.4	0.07	2.84	17	3.71	2.43	1.65	0.18	3.28	1.2
#5	5.6	0.1	7.74	24	7.57	4.79	1.52	0.31	3.30	2.2
#6	7.2	0.09	7.44	24	8.35	4.62	0.78	0.43	3.22	2.4
#7	5.9	0.1	7.26	20	8.32	6.63	1.74	0.28	2.79	2.2
#8	5.5	0.06	4.44	12	5.33	5.06	1.05	0.11	3.16	0.9
#9	5.6	0.1	4.8	28	6.01	3.23	1.68	0.25	3.28	1.9
#10	5.9	0.09	4.64	9	5.64	1.99	2.04	0.16	3.33	1.3
#11	5.8	0.04	3.69	8	6.39	3.21	0.42	0.02	2.42	0.6
#12	5.8	0.07	4.15	16	9.24	2.97	0.24	0.06	3.30	0.6
#13	6.4	0.09	12.17	11	23.46	2.05	1.18	0.20	3.25	1.8
#14	5.7	0.09	2.66	14	24.53	3.48	0.57	0.01	2.58	0.4
#15	6.3	0.13	8.67	21	34.3	1.85	1.38	0.22	3.25	1.9
#16	6.6	0.07	3.96	5	41.29	1.89	0.81	0.03	3.03	0.5

Note: \*AWCD = Average well color development.

components resulted from laboratory analyses (Table 1). Bacterial activity (determined by the values of AWCD at 72 h of incubation time) was significantly related to EC ( $p = 0.000$ ), SOM ( $p = 0.012$ ), and  $\text{NO}_3\text{-N}$  ( $p = 0.005$ ). A quadratic model most accurately represented the relationship between bacterial activity and SOM, although the model fitting was quite weak with  $R^2$  values of 0.18. Linear models were the most accurate representation of the positive relationship between bacterial activity and EC and  $\text{NO}_3\text{-N}$ , although the relationships were also weak with  $R^2$  values of 0.30 and 0.16, respectively (Table 2). There were no significant relationships between the activity of the bacterial communities and soil pH, SOC, CEC, and Ca:Mg ratio with the values of  $p > 0.05$ .

Fungal activity (determined by the values of turbidity at 72 h of incubation time) was significantly related to SOM ( $p = 0.023$ ),  $\text{NO}_3\text{-N}$  ( $p = 0.001$ ) and EC ( $p = 0.043$ ), whereas the relationships between fungal activity and soil pH, EC, SOC, CEC and Ca:Mg ratio were not significant ( $p > 0.05$ ). Similar to bacteria, the relationship between fungal activity and SOM was mostly accurately represented in a quadratic model, although the relationship was fairly weak with  $R^2$  value of 0.44, whereas the positive relationships between fungal activity and  $\text{NO}_3\text{-N}$  and EC were accurately represented in linear models, although the

relationships were weak with the  $R^2$  values of 0.59 and 0.26, respectively (Table 2).

A weak positive relationship was shown between bacterial activity and EC ( $Y = -0.0647 + 13.86X$ ,  $R^2 = 0.14$ ) (Figure 1), and between fungal activity and EC ( $Y = -0.077 + 3.096X$ ,  $R^2 = 0.26$ ) (Figure 2). At the lowest level of EC ( $0.04 \text{ dS m}^{-1}$ ), bacterial activity and fungal activity in soil were lower, indicated by the AWCD value of less than 0.5 absorbance level (abs.) units for bacterial community and less than 0.1 abs. units for fungal community. The activity of bacteria increased slowly to the level between 1.5 and 2.0 abs. units when the level of EC increased up to  $0.13 \text{ dS m}^{-1}$ , whereas fungal activity increased sharply to the level of 0.3 abs. units when EC value increased up to  $0.13 \text{ dS m}^{-1}$ .

A quadratic relationship was observed between bacterial activity and SOM ( $Y = -0.25 + 0.44X - 0.03X^2$ ,  $R^2 = 0.18$ ) (Figure 1), and between fungal activity and SOM ( $Y = -0.336 + 0.167X - 0.011X^2$ ,  $R^2 = 0.44$ ) (Figure 2). Soils containing lower levels of SOM had lower bacterial and fungal activity. Activity of these two functional groups increased and reached a peak when SOM content was at a level between 6% and 8%. Beyond 8% SOM, the activity of these two groups declined again.

A positive linear relationship was also observed between bacterial activity and  $\text{NO}_3\text{-N}$  ( $Y = 0.56 +$

Table 2. Regression models predicting bacterial and fungal activity as a function of soil chemical characteristics.

Variable	Bacteria		Fungi		Regression Model tested
	$R^2$	$p$	$R^2$	$p$	
Soil pH	0.05	0.346	0.17	0.304	Quadratic
EC ( $\text{dS m}^{-1}$ )	0.30	0.0001	0.26	0.043	Linear
SOM (%)	0.18	0.012	0.44	0.023	Quadratic
$\text{NO}_3\text{-N}$ ( $\text{mg L}^{-1}$ )	0.16	0.005	0.59	0.001	Linear
CEC ( $\text{meq } 100 \text{ g}^{-1}$ )	0.06	0.093	0.14	0.155	Linear
Ca:Mg ratio	0.001	0.805	0.12	0.182	Linear

Note:  $R^2$  = the coefficient of determination;  $p$ -value = calculated probability.

Table 3. Regression models predicting bacterial and fungal diversity (Shannon index) as a function of soil chemical characteristics.

Variable	Bacteria		Fungi		Regression Model tested
	$R^2$	$p$	$R^2$	$p$	
Soil pH	0.02	0.637	0.05	0.727	Quadratic
EC ( $\text{dS m}^{-1}$ )	0.15	0.006	0.39	0.010	Linear
SOM (%)	0.14	0.030	0.47	0.016	Quadratic
$\text{NO}_3\text{-N}$ ( $\text{mg L}^{-1}$ )	0.16	0.005	0.45	0.005	Linear
CEC ( $\text{meq } 100 \text{ g}^{-1}$ )	0.02	0.350	0.05	0.401	Linear
Ca:Mg ratio	0.03	0.218	0.04	0.451	Linear

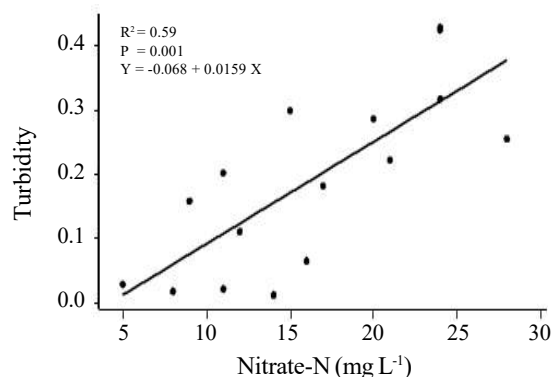
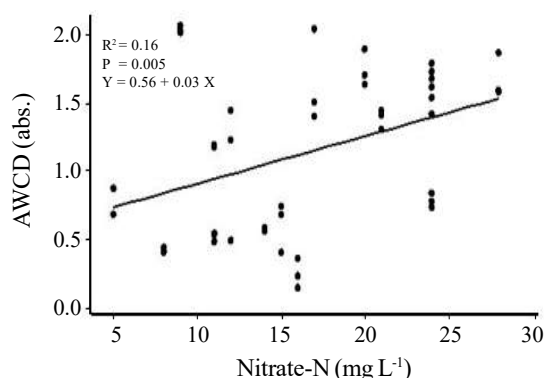
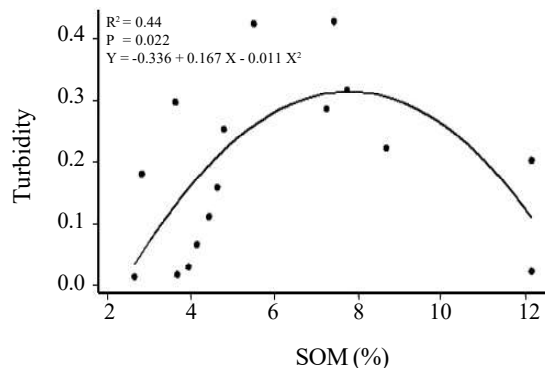
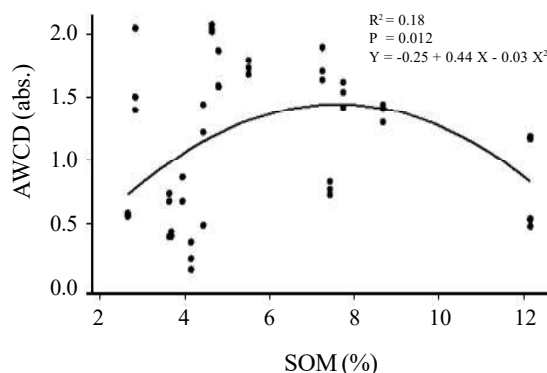
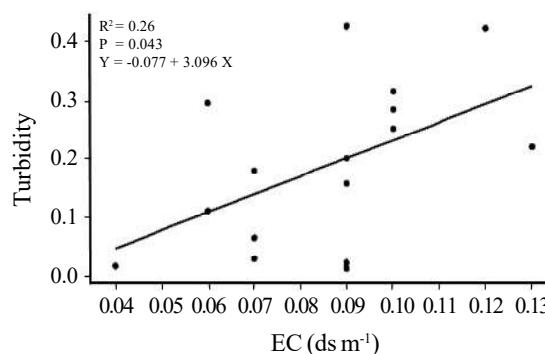
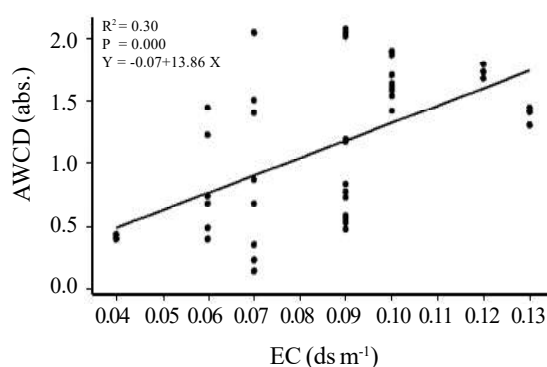


Figure 1. The relationships between EC, SOM, Nitrate-N and bacterial activity with statistical significance of  $p < 0.05$ .

Figure 2. The relationships between EC, SOM, Nitrate-N and fungal activity with statistical significance of  $p < 0.05$ .

0.03X,  $R^2 = 0.16$ ) (Figure 1), and between fungal activity and  $\text{NO}_3\text{-N}$  ( $Y = -0.068 + 0.0159X$ ,  $R^2 = 0.59$ ) (Figure 2). Soils with lower  $\text{NO}_3\text{-N}$  content had lower bacterial and fungal activity. Activity of bacteria and fungi increased when the level of  $\text{NO}_3\text{-N}$  increased.

#### Soil Bacterial and Fungal Diversity in Response to Soil Chemical Factors

The regression analyses using CLPP data revealed a significant relationship between bacterial diversity and EC ( $p = 0.006$ ), SOM ( $p = 0.030$ ),

and  $\text{NO}_3\text{-N}$  ( $p = 0.005$ ). Bacterial diversity was not significantly correlated to soil pH, SOC, CEC, and Ca:Mg ratio with  $p > 0.05$ . A similar pattern was observed when assessing fungal diversity, which showed a significant relationship with EC ( $p = 0.010$ ), SOM ( $p = 0.016$ ), and  $\text{NO}_3\text{-N}$  ( $p = 0.005$ ). Significant relationships between bacterial and fungal diversity and SOM were best described by quadratic models, whereas significant relationships between EC and bacterial and fungal diversity and between  $\text{NO}_3\text{-N}$  and bacterial and fungal diversity were best described using linear models (Table 3).

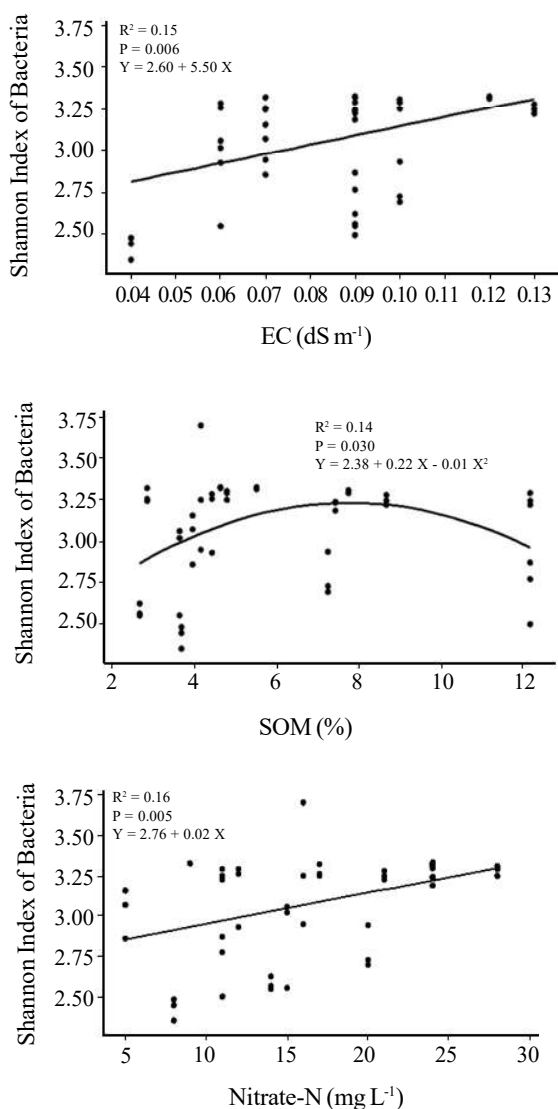


Figure 3. The relationships between EC, SOM, Nitrate-N and bacterial diversity with statistical significance of  $p < 0.05$ .

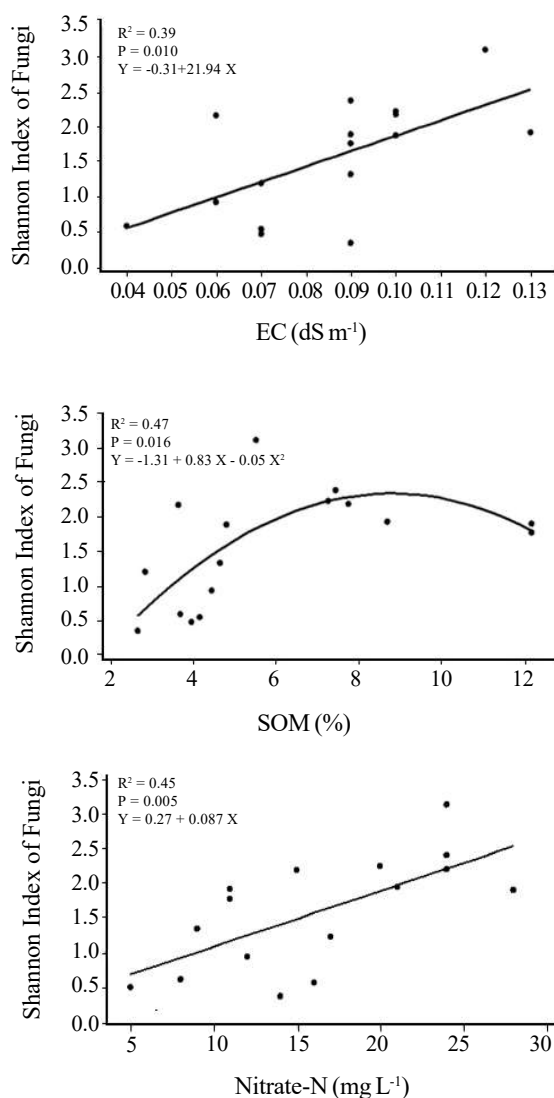


Figure 4. The relationships between EC, SOM, Nitrate-N and fungal diversity with statistical significance of  $p < 0.05$ .

The values of EC across the 16 sites were below 0.15 which were categorised as low and reflecting that the soils are non-saline (Cass 1998). Despite being low in EC content, bacterial and fungal diversity varied in the 16 soils. At the lowest EC (0.04 dS m<sup>-1</sup>) bacterial diversity was also low ( $H < 2.50$ ) in soil. Bacterial diversity increased progressively as the level of EC in soil increased (Figure 3). Similarly, fungal diversity was lower ( $H = 0.5$ ) in soils with a lower EC. The diversity of the fungal community continuously increased by five-fold as the EC increased up to 0.13 dS m<sup>-1</sup> (Figure 4).

In relation to the availability of SOM, bacterial diversity was moderate ( $2.50 < H < 3.0$ ) at lower

SOM content of between 2% and 4%. When SOM level was between 6% and 8%, the diversity of bacteria gradually increased and reached a peak ( $H > 3.0$ ). Thereafter, bacterial diversity declined (SOM 10% – 12%) (Figure 3). Meanwhile, fungal diversity was lower when SOM was between 2% and 4%. The diversity of the fungal community increased 4-fold when the level of SOM increased up to between 6% and 8%, and then declined when SOM > 10% (Figure 4).

At the lowest level of NO<sub>3</sub>-N, bacterial diversity was moderate ( $2.75 < H < 3.0$ ) and gradually increased as NO<sub>3</sub>-N increased (Figure 3). Meanwhile, lower fungal diversity ( $0.5 < H < 1.0$ ) was found in soils with the lowest NO<sub>3</sub>-N content

(Figure 4). The diversity of fungal community increased sharply when the level of  $\text{NO}_3\text{-N}$  increased.

## DISCUSSION

Bacterial and fungal diversity and activity were used as measures to assess microbial responses to soil chemical factors under varying soil conditions. The present study found that there was a significant quadratic relationship between soil organic matter and bacterial and fungal activity and diversity. The availability of soil organic matter influenced the activity and diversity of the soil microbial community, with bacterial activity and diversity higher in soils containing high SOM in the range of 4% – 8%. This was similar for fungal activity and diversity that was higher in soils containing high SOM in the range 6% and 8%. However, bacterial and fungal activity and diversity did not increase in soils containing soil organic matter levels greater than 8%. The results are broadly consistent with previous studies that have demonstrated that soil microbial diversity was positively correlated with SOM (Cookson, Murphy and Roper, 2008; J Garcia-Pausas and Paterson, 2011; Brennan and Acosta-Martinez 2017), although the decline with high SOM levels is less widely reported. In an experiment involving manipulation of SOM, a non-linear relationship was also found by Stark *et al.* (2008) when studying the effect of varying rates of C substrate on soil microbial biomass and activity.

A significant linear relationship also occurred between N compounds and bacterial and fungal activity and diversity. It was noted that the presence of  $\text{NO}_3\text{-N}$  less than 10 ppm (low level) in soil had resulted in low activity and diversity of these two microbial groups. A positive linear relationship between soil N compounds and the activity and diversity of bacteria and fungi was found. It appeared that higher activity and diversity of bacteria and fungi were related to the presence of moderate levels of  $\text{NO}_3\text{-N}$  in the soils. This was likely linked with the role of bacterial and fungal community in N mineralisation. According to Kibblewhite *et al.* (2008), nitrogen cycling affects the delivery of ecosystem services driven by soil microbial community, so soil microbial activity may frequently be limited by N content in soil.

In previous studies, however, it was noted that there were inconsistent relationships between N compounds and activity and diversity of microbial community. Bengtsson *et al.* (2003) have found that microbial biomass and activity were weakly correlated with N mineralisation. Shi *et al.* (2006)

suggested that the lack of correlation between microbial activity and the N mineralisation process was likely a result of changes in soil microbial composition. Also, in a study on the application of nitrogen fertilizer, Lupwayi *et al.* (2012) have found that the recommended rate of N application (50 – 80 kg ha<sup>-1</sup>) had no significant effects on soil microbial biomass and bacterial diversity.

In the present study, soil pH, CEC and soil Ca:Mg ratio had no significant effects on bacterial and fungal diversity. This result indicated that any effects of these chemical factors on the differences in soil microbial communities among the sites were small. Yet, Fierer and Jackson (2006) and Nicol *et al.* (2008) have found a strong correlation between soil pH and bacterial diversity. The diversity of soil bacterial communities on a global scale are higher in neutral soils and lower in acidic soils (Fierer and Jackson, 2006). Lauber *et al.* (2008) and Wakelin *et al.* (2008) also concluded that soil pH is involved in shaping the diversity of bacterial community, because bacterial communities often co-vary with shift of soil pH, whereas diversity of fungal communities is mainly affected by a shift in soil nutrient status. However, some other previous studies have found a poor correlation between fungal community and soil pH by considering that fungal communities exhibit a wider range of pH for optimal growth (Lauber *et al.* 2008; Rousk *et al.* 2010).

The present study found a weak correlation, or no correlation, between bacterial and fungal activity and diversity and CEC, and between bacterial and fungal activity and diversity and Ca:Mg ratio. This was likely because the heterogeneity of the data of chemical parameters among sites was relatively high, whereas the response data of activity and diversity of bacteria and fungi were relatively homogenous among sites. Stark *et al.* (2008) explain that poor correlations between soil microbial activity and soil chemical characteristics can occur due to the masking of responses by other soil and environmental factors that have greater effects on soil microbial characteristics.

## CONCLUSIONS

Of all soil chemical characteristics measured in the current study, the study provides evidence that SOM plays an important role in affecting soil microbial activity and diversity under either untreated (natural) or treated conditions, and therefore this soil component cannot be ignored. Despite having significant relationships with soil microbial activity and diversity, soil salinity (EC) and N compound

effects can be different among sites and therefore can possibly result in weak correlations with soil microbial activity and diversity. The chemical characteristics such as EC and N compounds together with other chemical properties (*i.e.* pH, CEC, Ca:Mg ratio) can therefore be ruled out.

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